Regulating the TRAIL of Destruction: How A20 Protects Glioblastomas from TRAIL-Mediated Death

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Summary: In this issue of Cancer Discovery, Bellail and colleagues unravel how overexpression of the ubiquitin-modifying enzyme A20 results in TNF-related apoptosis-inducing ligand (TRAIL) resistance in glioblastoma. After TRAIL receptor stimulation, A20 mediates the polyubiquitination of RIP1 at the TRAIL receptor tail, resulting in the interaction of the polyubiquitin chain to pro caspase-8 that is recruited to the TRAIL-bound receptors. The inability of ubiquitin-bound pro caspase-8 to be dimerized and activated prevents the execution of the apoptotic program. Cancer Discovery. 2(2); 112–4. ©2012 AACR.

Commentary on Bellail et al., p. 140 (8).

A20, however, is best known as a potent anti-inflammatory protein; it inhibits NF-κB by various mechanisms. It harbors an N-terminal ovarian tumor (OTU) deubiquitination domain and 7 C-terminal zinc finger (ZnF) motifs (4). The OTU domain removes lysine (K) 63-linked ubiquitin chains from multiple proteins, including receptor-interacting protein 1 (RIP1/RIPK1), whereas the ZnF region was shown to add K48-linked polyubiquitin chains to RIP1, promoting its proteasomal degradation (4, 5).

RIP1 itself is also an important regulator of cell death, and its ubiquitination state determines whether it functions as a prosurvival scaffold molecule or a kinase that promotes cell death. K63-linked ubiquitinated RIP1 promotes activation of prosurvival pathways such as NF-κB (6). However, in the absence of a functional NF-κB signaling pathway or in situations of reduced ubiquitination of RIP1, the kinase is a potent proapoptotic protein. In addition, when apoptotic cell death is blocked by caspase inhibitors, RIP1 is essential for the execution of caspase-independent necroptosis (7).

In this issue of Cancer Discovery, Bellail and colleagues (8) unravel how overexpression of A20 mediates TRAIL resistance in glioblastoma. They observe that in TRAIL-sensitive tumors with low A20 expression, TRAIL-R2 associates with TNF receptor-associated factor 2 (TRAF2) and RIP1 in a preligand assembly complex. After treatment with TRAIL, FADD and pro caspase-8 are recruited to the death receptor tail, forming the DISC, resulting in activation and cleavage of caspase-8 and subsequent apoptosis (Fig. 1A). In TRAIL-resistant glioblastomas, A20 is overexpressed, and TRAIL-R2 is associated with TRAF2, RIP1, and A20 in the preligand assembly complex. Upon TRAIL stimulation, the ZnF motif of A20 mediates K63-linked polyubiquitination of RIP1. Although both FADD and pro caspase-8 are recruited to the DISC, the interaction of the polyubiquitin chain with the p18 subunit of pro caspase-8 inhibits its dimerization, cleavage, and activation, preventing execution of the apoptotic program (Fig. 1B).
they showed that RIP1 was ubiquitinated through both K48-linked ubiquitination by A20. Using reconstitution assays, they demonstrated that K63-linked ubiquitin chains significantly inhibited caspase-8 cleavage in cells, followed by TRAIL stimulation and immunoprecipitation of RIP1, corroborated K63-linked RIP1 ubiquitination after treatment with TRAIL.

To determine whether RIP1, A20, or TRAF2, which were all present in the preligand assembly complex and the DISC of TRAIL-resistant glioblastoma cell lines, contributed to TRAIL resistance, the authors knocked down expression of these proteins with siRNA. Knockdown of either RIP1 or A20, but not TRAF2, resulted in the cleavage of caspase-8 at the TRAIL-R2 DISC, implying that both RIP1 and A20 are required for inhibition of caspase-8 cleavage in the TRAIL-R2 DISC.

Next, they mapped the motif in A20 responsible for inhibition of caspase-8 cleavage by transfecting A20 point mutants in A20-deficient, TRAIL-sensitive cells. Both wild-type and OTU mutant A20 conferred TRAIL resistance in LN71 cells, whereas the ZnF4 mutant (C624A, C627A) did not. These results imply that in the presence of RIP1, the ZnF4 motif of A20 mediates TRAIL resistance in glioblastoma. This provides an additional mechanism by which A20 can mediate TRAIL resistance that was previously reported by inhibition of the protease domain of caspase-8, but not the catalytic site itself, directly bound K63-linked ubiquitin chains. In addition, they demonstrated that K63-linked polyubiquitin chains significantly inhibited caspase-8 dimerization and cleavage. Finally, they showed that A20, RIP1, and TRAIL-R2 were present in the TRAIL-R2 preligand assembly complex of TRAIL-resistant tumor-initiating cells from glioblastomas surgically removed from patients. After treatment with TRAIL, FADD and caspase-8 were recruited to the DISC, where K63-linked polyubiquitin chain-conjugated RIP1 was detected. The tumor-initiating cells could be sensitized to TRAIL by A20 siRNA and overexpression of the A20 ZnF4 mutant, the latter acting most likely through dominant-negative effects on wild-type A20.

Although these biochemical studies shed light on the molecular events that may underpin resistance of glioblastoma...
cells to TRAIL-mediated apoptosis, several questions still remain. First, upon downregulating A20 via the use of siRNA in TRAIL-resistant glioblastoma cells, RIP1 dissociates from the DISC upon TRAIL treatment, suggesting that FADD competes with RIP1 for death domain binding in the DISC. Therefore, how is RIP1 stabilized in the DISC of TRAIL-resistant glioblastoma? Does ubiquitinated RIP1 stabilize its interaction with the death domains of TRAIL-R2, allowing its binding to procaspase-8 once the latter is recruited? Is the presence of A20 required for RIP1 to be associated in the DISC, or does the binding of the polyubiquitin chain to caspase-8 stabilize RIP1?

Second, TRAF2 is a molecule not normally associated with the TRAIL-R2 preligand assembly complex or DISC but is recruited to multiple signaling complexes that activate NF-κB. Upon treatment with TNF-α, TRAF2 is recruited to TNFR1 via the adaptor protein TRADD (9), where it mediates K63-linked polyubiquitination of RIP1 (5). So what is TRAF2 doing in the TRAIL-R2 preligand assembly complex and DISC and how is it associated with TRAIL-R2? TRAF2 downregulation cannot sensitize TRAIL-resistant glioblastoma cell lines to TRAIL, but what is the effect of TRAF2 downregulation in TRAIL-sensitive glioblastomas? Is TRAF2 required for caspase-8 activation?

Finally and of great importance, because A20 has now been described to function as a positive and negative regulator of K63-linked ubiquitination of RIP1 after TNF-α (5) and TRAIL stimulation (8), how are these different functions regulated? Are there differences in composition of the TNFR1 and TRAIL-R2 signaling complexes that mediate these functions of A20 or is it dependent on the cell type studied? In addition and further complicating the role of A20 as a ubiquitin-modifying enzyme, both the OTU domain and the ZnF motif possess E2-binding activity, and the A20 ZnF4/ZnF7 motifs themselves bind K63-linked polyubiquitin chains interacting to and inhibiting the function of NEMO, a component of the IkB kinase/NF-κB signaling pathway (4, 10).

Although these molecular/mechanistic questions require additional experimentation to fully decipher the answers, the potential clinical impact of the current studies in terms of translating the findings into treatment options for glioblastoma is tantalizing. Is downregulation/inhibition of A20 a viable strategy to sensitize glioblastomas to TRAIL-receptor agonist therapies? Efforts to pharmacologically inhibit ubiquitin-ligases and deubiquitinating enzymes have already commenced (e.g., see refs. 11 and 12), so specifically inhibiting the enzymatic activity of A20 is a formal possibility. Moreover, the development of small molecule inhibitors of protein–protein interactions such as MDM2–p53 and Bcl-2–Bax/Bak provides irrefutable evidence that proteins previously considered “undruggable” can indeed be molecularly targeted. This provides an optimistic outlook for the development of novel therapeutic strategies involving recombinant TRAIL or agonistic anti-TRAIL receptor antibodies in conjunction with small molecule inhibitors of molecules such as A20 for the treatment of TRAIL-resistant tumors such as glioblastoma.

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No potential conflicts of interest were disclosed.

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